

Effect of carbohydrate supplementation on plasma glutamine during prolonged exercise and recovery.

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Effect of Carbohydrate Supplementation on Plasma Glutamine During Prolonged Exercise and Recovery

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■ Muscle glycogen and glucose have been suggested to be carbon-chain precursors for glutamine synthesis in skeletal muscle. Therefore, the aim of the present study is to investigate whether carbohydrate supplementation affects plasma glutamine and other amino acids during exercise and 7 h of recovery. Eight well-trained subjects cycled at an alternating workload of 50 and 80% W_{\max} until exhaustion (59 to 140 min). During the exercise bout the subjects received either water (control) or a carbohydrate (CHO) drink (83 g $\text{CHO} \cdot \text{l}^{-1}$, 2 ml $\cdot \text{kg}^{-1}$ per kg body weight every 15 min). Plasma glutamine concentration appeared not to be affected by exercise, as a significant increase was only observed at some points in time during the control test. During recovery, however, plasma glutamine concentration decreased from 682 ± 24 and $685 \pm 19 \mu\text{mol} \cdot \text{l}^{-1}$ at exhaustion to 552 ± 19 and $534 \pm 12 \mu\text{mol} \cdot \text{l}^{-1}$ after 2 h of recovery for the control and CHO test, respectively. Plasma glutamine concentration returned to pre-exercise values after 7 h of recovery. Alanine concentration increased during exercise in both tests. During the recovery period the concentration of alanine (48%), and total amino acids (23%) decreased below the pre-exercise level. The plasma alanine and the total amino acid concentration was still suppressed after 7 h of recovery. In conclusion, carbohydrate supplementation had neither an effect during exercise nor during recovery on the concentration of plasma glutamine or other amino acids. Exercise, however, causes a substantial decrease in the plasma amino acid concentration during recovery.

■ Key words: Ammonia, amino acids.

Introduction

Glutamine is the most abundant amino acid in human plasma and muscle. In muscle glutamine accounts for 60% of the total intracellular muscle free amino acid pool (excluding the non-protein amino acid taurine) (2). In the postabsorptive state glutamine (48%) and alanine (32%) together account for 80% of the amino acids released from skeletal muscle (19), implying that glutamine with two N-atoms per molecule is dominant for the amino acid N-release from muscle. In the 4 hour period after the ingestion of a mixed meal the dominance of glutamine in carrying N out of skeletal muscle is even greater. Glutamine then accounts for 71% of the amino acid release and 82% of the N release from muscle (9). As the relative occurrence of glutamine in muscle protein is only 10–15% this indicates that the glutamine *de novo* synthesis in muscle is substantial 24 hours per day. Tracer studies in man (7,22) also indicate that production rates of glutamine are higher than of any other amino acid and that glutamine is more important than alanine as a vehicle for transporting amino acid carbon and nitrogen from muscle through the plasma to the sites of oxidation. Recent tracer studies also suggest that glutamine is more important than alanine as a gluconeogenic precursor in postabsorptive humans (22).

The biochemical pathways in muscle that contribute to glutamine production in skeletal muscle are only partly known. In the postabsorptive state the uptake of glutamate from the circulation accounts for 18–65% of the glutamine production (mean value of 5 studies is 40%) (9,10,19,22,29). Part of the glutamine production in the postabsorptive state also results from the net breakdown of muscle protein (22). Protein degradation not only produces glutamine directly, but also leads to the production of branched-chain amino acids, glutamate, aspartate and asparagine. The carbon skeletons of these amino acids have all been shown to be used for *de novo* synthesis of glutamine in incubated rat muscles (4,30). Sir Hans Krebs (16) suggested that the carbon skeletons of muscle glycogen and blood glucose are also used for synthesis of TCA cycle intermediates and glutamine. The reaction used for conversion of glycolytic 3-carbon molecules to TCA cycle intermediates either is reversal of the phosphoenolcarboxykinase reaction (16) or pyruvate carboxylase (8). Exercise leads to an increase in glutamine release from muscle (10,27,29). However, this increase was only temporary during 90 min of one leg exercise at 60–65% W_{\max} (29). A decrease in plasma glutamine was ob-

served during and following 4 hours of treadmill running at 40% $\dot{V}O_2$ max leading to glycogen depletion in untrained subjects (25). Such decreases in plasma glutamine may be important as it has been suggested to be associated with immune system impairment in overtrained athletes (24). Here we investigated whether decreases in plasma glutamine and other amino acids were also observed during and following prolonged high intensity exercise with elite cyclists and whether glucose ingestion could prevent such decreases in line with the suggestion that glucose is a carbon-chain precursor for glutamine synthesis.

Material and Methods

Subjects

Eight healthy and highly trained male cyclists, competing at national and international level participated in the study (4 professional cyclists and 4 amateur cyclists from the Dutch national team). The subjects were informed about procedures and possible risks involved before giving their voluntary consent to participate. Their age, weight, and length (mean \pm SD) were 20.9 ± 2.4 yr, 73.3 ± 4.6 kg, and 1.82 ± 0.04 m, respectively.

Protocol

The subjects were studied while exercising on an electromagnetically braked cycle ergometer (Lode, The Netherlands) at a freely chosen, power output independent pedalling rate (60–120 revolutions \cdot min $^{-1}$). Before the experiment (on a separate day) each subject performed a maximal workload test to determine their maximal work output (W_{max} , $407 \text{ W} \pm 20$) as described previously (17). The subjects were studied during exercise until exhaustion and 7 hours of recovery on two separate occasions, first the control test when only water was ingested followed by the carbohydrate supplementation test. The study was not randomized since carbohydrate supplementation is expected to improve performance and thus subjects would have exercised longer thereby partly abolishing the effects of carbohydrate supplementation during the recovery phase. Therefore, time to exhaustion was determined in the control trial and repeated in the carbohydrate trial. Although we cannot exclude an order effect these effects are expected to be minimal as the subjects were all familiar with cycle ergometer exercise at these intensities in the laboratory. The athletes are used to performing this kind of intense exercise daily and, therefore, at least three days of recovery between the test appears to be more than sufficient for recovery. Therefore, an order effect due to training or insufficient recovery appears to be small. Both tests were performed within 2 weeks of assessment of W_{max} . During the test days the subjects reported to the laboratory at 7.30 a.m. after an overnight fast. They were allowed to eat a standardized breakfast that consisted of two sandwiches with marmalade and water or tea. The exercise bout started at 8.45 with a warming-up period of 5 min at 100 W followed by 5 min at 50% W_{max} . The subjects then cycled at alternating exercise intensities in blocks of 6 min at 80% W_{max} and 3 min at 50% W_{max} until exhaustion was reached. Exhaustion was defined as the inability to maintain a pedalling rate above the 60 revolutions \cdot min $^{-1}$ despite encouragement. During the control test the subjects had to drink 4 ml \cdot kg $^{-1}$ body weight water in the warming-up period and then every 15 min 2 ml \cdot kg $^{-1}$ body weight of water. After the exercise bout

they were only allowed to drink water or tea without sugar. The test with carbohydrate ingestion was performed at least 3 days after the control test. The standardized breakfast at 7.30 a.m. was supplemented with 0.5 liter of a carbohydrate drink (166 g CHO \cdot l $^{-1}$, Perform, Wander Ltd., Bern, Switzerland) replacing water or tea. The exercise bout started at 8.45 and was performed as described for the control test. During the warming-up period the subjects had to drink 4 ml \cdot kg $^{-1}$ body weight of the carbohydrate drink (83 g CHO \cdot l $^{-1}$) and then every 15 min 2 ml \cdot kg $^{-1}$ body weight of the same CHO-drink, water was provided *ad libitum* during the whole test. Exercise was terminated for each subject at the exact moment that exhaustion was reached in the control test. During the recovery phase they were only allowed to drink water or tea without sugar.

Analysis

Blood was sampled during the exercise bout and 7 hour recovery from a forearm vein using a catheter kept patent by flushing with saline. Samples were obtained just before warming-up and at the end of the 80% W_{max} blocks at 34, 61, 88, 124 min, and at the moment of exhaustion. During the recovery period blood samples were obtained after 20, 40, 60, 120, 180, 300 and 420 min of recovery. Heparinized blood was centrifuged immediately to obtain plasma; aliquots of plasma were frozen for analysis of glucose, lactate, and ammonia. Plasma for the analysis of amino acids was deproteinized with sulfosalicylic acid (6 mg \cdot 100 μ l $^{-1}$ plasma) and analyzed by HPLC (Pharmacia, The Netherlands) (28) and stored at -80°C . Ammonia was measured using a modification of the enzymatic determination with glutamate dehydrogenase (14) performed on a COBAS BIO analyzer (Roche, The Netherlands). Plasma lactate and glucose were analyzed with standard enzymatic techniques on a COBAS BIO analyzer.

Statistics

All data are means \pm SEM. Significant differences were determined between related samples of the same subject in the two tests and differences of the same subject at different times during exercise and recovery with one-way repeated measures analysis of variance (ANOVA), location of significance was determined with the Fisher protected least significance difference test. Statistical significance was set at $P < 0.05$.

Results

Performance

Exercise time to exhaustion with the alternating workload protocol of 6 min at 80% W_{max} and 3 min at 50% W_{max} varied from 59 to 140 min. Plasma lactate concentrations were higher in the subjects with the relatively short exercise times. Seven of the eight subjects were able to continue exercise with carbohydrate ingestion for the same period of time as they had reached in the control test.

Plasma metabolites

Plasma glucose concentration during the exercise bout was similar for both tests. During the recovery period, however, plasma glucose concentration increased the first 40 min in the test with carbohydrate ingestion whereas the plasma glu-

cose concentration decreased to pre-exercise concentrations in the control test (Fig. 1). No significant differences were observed between the test with carbohydrate supplementation and control test for plasma ammonia and lactate concentration during exercise and recovery (Fig. 1). Plasma ammonia gradually increased with exercise duration. Plasma lactate increased fourfold compared to resting level during the first 30 min of exercise but then remained constant until exhaustion (Fig. 1). During the first hour of recovery a sharp fall was observed in plasma ammonia and lactate concentration and within 40 min of recovery their concentrations returned to pre-exercise values. Plasma glutamine concentration increased significantly during exercise in the control test and alanine increased in both tests (Fig. 2). The concentration of plasma glutamine and alanine declined rapidly during the initial phase of recovery and reached the lowest concentration after 2 hours. Both glutamine and alanine concentrations decreased below pre-exercise concentrations but glutamine returned to pre-exercise concentrations after 5 to 7 hours of recovery whereas alanine remained low even after 7 hours of recovery (Fig. 2). Plasma glutamate increased during the first 20 to 40 min of recovery. The pre-exercise BCAA concentration in the control test was significantly higher than in the carbohydrate supplementation test. The BCAA decreased with exercise and during the first hour of recovery. Plasma BCAA concentration in the control test remained below the pre-exercise con-

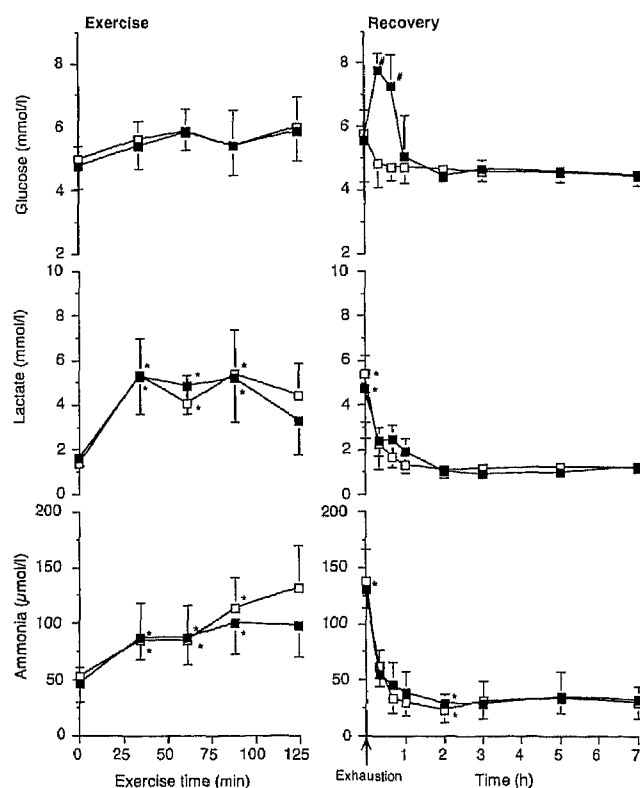


Fig. 1 Plasma glucose, lactate and ammonia in 8 highly trained subjects during exercise and recovery. Values are means \pm SEM obtained in the carbohydrate supplementation test (closed squares) and the control test (open squares). Values during exercise are of 8 subjects at 34 min, of 7 subjects at 61 min, of 5 subjects at 88 min and of 2 subjects at 124 min. * Significant differences from the pre-exercise concentration during exercise as well as recovery, # significant differences between the carbohydrate- and control test.

centration but in the carbohydrate supplementation test plasma BCAA concentration returned to pre-exercise concentration within 5 hours. The absolute BCAA concentration during recovery was equal for both tests (Fig. 2). The pattern of total plasma amino acid concentration resembles that of alanine and glutamine since these amino acids together account for about 40–50% of the total amino acid concentration.

Discussion

The new finding in this study is that the concentration of most plasma amino acids shows a remarkable (10–25%) decrease after moderate to high intensity exercise performed in blocks alternating at 80% and 50% W_{max} simulating intense cycling competition of relatively short duration (1–2 hours). The

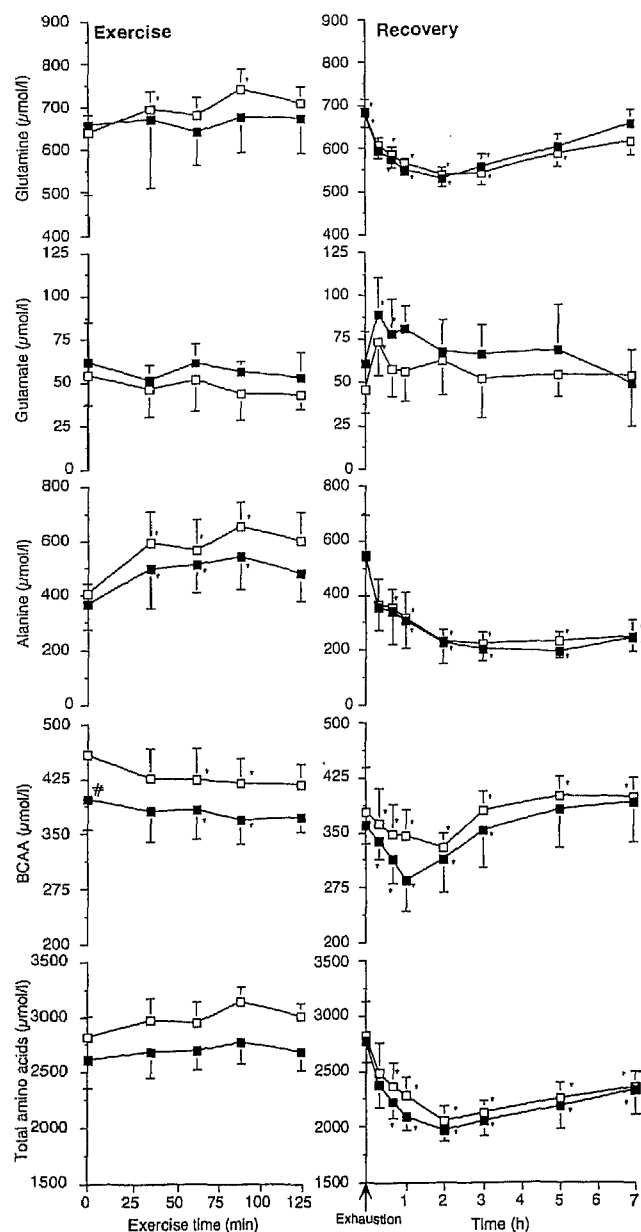


Fig. 2 Plasma amino acid concentration during exercise and recovery. Details are given in legend to Fig. 1.

mechanism behind the post-exercise decrease in the plasma amino acid concentration is not clear. One possibility is a temporary increase of muscle protein synthesis rates and net muscle protein deposition as has been observed during recovery from 4 hours of cycling exercise at 40% $\dot{V}O_2\text{max}$ (5) during recovery from resistance exercise (6). It also cannot be excluded that an increased clearance of plasma amino acids by other tissues (e.g. gut, liver) contributes to the reduction of plasma amino acid concentration.

The post-exercise reduction of the plasma concentration is also seen for alanine and glutamine. These amino acids are special as they are rapidly synthesized *de novo* in human skeletal muscle at rest from branched-chain amino acids (BCAA) and carbon-chain precursors as pyruvate and α -ketoglutarate both after consumption of a protein containing meal and in the postabsorptive state (11,12,22). In the BCAA aminotransferase reaction the amino group is donated to α -ketoglutarate ($\text{BCAA} + \alpha\text{-ketoglutarate} \leftrightarrow \text{BC} \alpha\text{-keto acids} + \text{glutamate}$). In the reaction catalyzed by glutamine synthase, glutamate may then react with ammonia to give glutamine the main non-toxic amino group carrier released by human skeletal muscle 24 hours a day. Alternatively, glutamate may donate the amino group to pyruvate to form alanine, a reaction catalyzed by alanine aminotransferase ($\text{glutamate} + \text{pyruvate} \leftrightarrow \text{alanine} + \alpha\text{-ketoglutarate}$). In the postabsorptive state muscle glutamate uptake from the circulation can account for 40% of the muscle glutamine production (mean value of 9, 10, 19, 22, 29). Exercise leads to significant change in the rate at which alanine and glutamine are released by skeletal muscle (1, 10, 11, 15, 29). Felig (11) was the first to report that the alanine output increased greatly with and in proportion to the exercise intensity and the rate of glycolysis and pyruvate accumulation. During prolonged exercise at moderate work load the release of both alanine and glutamine increased during the first 30 min and then gradually returned to resting values when exercise was continued for 90 min (29). This may indicate that the rate of alanine and glutamine production is reduced when the glycogen concentration in muscle is decreased towards exhaustion of prolonged exercise and maybe also following exercise.

The other major finding of this study is that oral ingestion of carbohydrates prior to and during exercise has no effect on the concentration of plasma glutamine, alanine and ammonia during exercise and cannot prevent the post-exercise decrease in plasma glutamine and other amino acids. This may imply that blood glucose is relatively unimportant as a precursor for muscle glutamine production during and following prolonged intense exercise in contrast to the original suggestion of Sir Hans Krebs (16).

After prolonged intense exercise the number of lymphocyte in the blood is reduced, and the function of natural killer cells is suppressed; furthermore, secretory immunity is impaired (23). During this time of immuno-suppression, referred to as "open window" phenomenon, the host may be more susceptible to microorganism by passing the first line of defense (23). Glutamine has more metabolic functions than any other amino acid and is considered as a conditionally essential amino acid in catabolic stress situation (18). Among other functions, glutamine is consumed by rapidly dividing cells such as cells of the immune system probably for both energy and as a metabolic precursor for cell replication (18). Newsholme and col-

leagues have linked decreases in plasma glutamine to a weakened immune response in heavily training and overtrained athletes (20, 21, 24), although experimental evidence for the existence of such a link is lacking. However, the clear parallelism between the time course of the reduction in glutamine concentration after intense exercise observed in this study and the "open window" phenomenon is remarkable. Recently, Rhode et al. (26) found in a time course of several days after a marathon race that serum glutamine concentration was correlated with changes in lymphokine activated killer cell activity but not with natural killer cell activity or proliferation (26). The practical message of this study is that similar decreases in plasma glutamine as observed in overtrained athletes also are seen in the 7 hour period following prolonged intense exercise in elite athletes on a training schedule. Further research is needed to investigate whether this decrease is related to the "open window" phenomenon and whether nutritional means can be used to prevent the decrease in plasma glutamine and the "open window" phenomenon in parallel. This study finally shows that carbohydrate ingestion during exercise does not prevent the post-exercise decrease in plasma glutamine and the "open window" phenomenon in parallel. This study finally shows that carbohydrate ingestion during exercise does not prevent the post-exercise decrease in plasma glutamine.

References

1. Ahlborg G, Felig P, Hagenfeld L, Hendler R, Wahren J. Substrate turnover during prolonged exercise in man. *J Clin Invest* 1974; 53: 1080–90
2. Bergström J, Fürst P, Hultman E. Free amino acids in muscle tissue and plasma during exercise in man. *Clin Physiol* 1985; 5: 155–60
3. Broberg S, Sahlin K. Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. *J Appl Physiol* 1989; 67: 116–22
4. Chang TW, Goldberg AL. The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. *J Biol Chem* 1978; 253: 3685–95
5. Carraro F, Stuart CA, Hartl WH, Rosenblatt J, Wolfe RR. Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am J Physiol* 1990; 259: E470–E476
6. Chesley A, McDougall JD, Tarnopolsky MA, Atkinson SA, Smith K. Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol* 1992; 73: 1383–8
7. Darmaun D, Matthews D, Bier D. Glutamine and glutamate kinetics in humans. *Am J Physiol* 1986; 251: E117–E126
8. Davis EJ, Spydevold Ø, Bremer J. Pyruvate carboxylase and propionylCoA carboxylase as anaplerotic enzymes in skeletal muscle mitochondria. *Eur J Biochem* 1980; 110: 255–62
9. Elia M, Schlatmann A, Goren A, Austin S. Amino acid metabolism in muscle and in the whole body of man before and after ingestion of a single mixed meal. *Am J Clin Nutr* 1989; 49: 1203–10
10. Eriksson LS, Broberg S, Björkman O, Wahren J. Ammonia metabolism during exercise in man. *Clin Physiol* 1985; 5: 325–36
11. Felig P. Amino acid metabolism in man. *Ann Rev Biochem* 1975; 44: 933–55
12. Goldberg AL, Chang TW. Regulation and significance of amino acid metabolism in skeletal muscle. *Fed Proc* 1978; 37: 2301–7
13. Golden MHN, Jahoor P, Jackson AA. Glutamine production rate and its contribution to urinary ammonia in normal man. *Clin Sci* 1982; 62: 29–305

14. Janssen MA, Van Berlo C, Van Leeuwen PAM, Soeters PB. The determination of ammonia in plasma and whole blood. In: Soeters PB, Wilson JHP, Meijer AJ, Holm E, editors. *Advances in Ammonia Metabolism and Hepatic Encephalopathy*. Amsterdam: Excerpta Medica 1988; 587–92
15. Katz A, Broberg S, Sahlin K, Wahren J. Muscle ammonia and amino acid metabolism during dynamic exercise in man. *Clin Physiol* 1987; 6: 365–79
16. Krebs HA. The role of chemical equilibrium in organ function. *Adv Enzym Reg* 1975; 15: 449–72
17. Kuipers H, Keizer H, Brouns F, Saris WHM. Carbohydrate feeding and glycogen synthesis in exercise in man. *Pflugers Arch* 1987; 410: 652–6
18. Lacey JM, Wilmore DW. Is glutamine a conditionally essential amino acid? *Nutr Rev* 1990; 48: 297–309
19. Marliss EB, Aoki TT, Pozefsky T, Most AS, Cahill GF. Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. *J Clin Invest* 1971; 50: 814–7
20. Newsholme EA, Newsholme P, Curi R. The role of the citric acid cycle in cells of the immune system and its importance in sepsis, trauma and burns. *Biochem Soc Symp* 1987; 54: 145–61
21. Newsholme EA, Parry-Billings M. Properties of glutamine release from muscle and its importance for the immune system. *JPEN* 1990; 14: 63–7
22. Nurjhan N, Bucci A, Perriello G, Stumvoll M, Dailey G, Bier DM, Toft I, Jenssen TG, Gerich JE. Glutamine: A major gluconeogenic precursor and vehicle for interorgan carbon transport in man. *J Clin Invest* 1995; 95: 272–7
23. Pedersen BK, Ullum H. NK cell response to physical activity: possible mechanism of action. *Med Sci Sports Exerc* 1994; 26: 140–6
24. Parry-Billings M, Budgett R, Koutedakis Y, Blomstrand E, Brooks S, Williams C, Calder PC, Pilling S, Baigrie R, Newsholme EA. Plasma amino acid concentrations in the overtraining syndrome: possible effect on the immune system. *Med Sci Sports Exerc* 1992; 24: 1353–8
25. Rennie MJ, Edwards RHT, Krywawych S, Davies CTM, Halliday D, Waterlow JC, Millward DJ. Effect of exercise on protein turnover in man. *Clin Sci* 1981; 61: 627–39
26. Rohde T, MacLean DA, Hartkopp A, Pedersen BK. The immune system and serum glutamine during a triathlon. *Eur J Appl Physiol* 1996; 74: 428–34
27. Sahlin KA, Katz A, Broberg S. Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *Am J Physiol* 1990; 259: C834–C841
28. Van Eijck HMH, Van der Heijden MAH, Van Berlo CMH, Soeters PB. Fully automated liquid chromatographic determination of amino acids. *Clin Chem* 1988; 34: 2510–13
29. Van Hall G, Saltin B, Van der Vusse GJ, Söderlund K, Wagenmakers AJM. Deamination of amino acids as a source for ammonia production during prolonged exercise. *J Physiol* 1995; 489: 251–61
30. Wagenmakers AJM, Salden HJM, Veerkamp JH. The metabolic fate of branched-chain amino acids and 2-oxo acids in rat muscle homogenates and diaphragms. *Int J Biochem* 1985; 17: 957–62

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